UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM:

To: Samantha Hulkower

From: Kevin Sweeney, Senior Entomologist

Date: March 8, 2012

Subject: PRODUCT PERFORMANCE DATA EVALUATION RECORD

DP barcode: 393428
Decision no.: 452547
Submission no: 900601
Action code: R310
Product Name: Attractive Toxic Sugar Bait
EPA Reg. No or File Symbol: 85125-E
Formulation Type: RTU bait
Ingredients statement from the label with PC codes included: (PC code: 011001) 4.00% Boric Acid
Application rate(s) of product: Trap density is not stated. The net contents of one cartridge are to be used but the amount in one cartridge is not stated.
Use Pattern: Bait application delivered by a trap for control of mosquitoes and sand flies. OCSPP Guidelines 810.3400 and 810.3500 to the extent that they apply.

I. Action Requested: Review two cited published studies, one original study, and the new label for the subject product.

II. Background: The registrant cited three studies. The published sand fly study reports the results of a field study that evaluated the application of bait solution (ATSB) applied to vegetation, barrier fencing, and in bait station traps for use against the sand fly, *Phlebotomus papatasi*. The second study examined the application of the formulation when used in traps in a controlled laboratory environment against three species of mosquitoes. The third study was a field study done in Florida against one mosquito species but included a treatment solution was 1.0w/v boric acid that was approximately the same as the candidate formulation.

This product was submitted in 2010 as EPA File Symbol 85125-R and withdrawn. Efficacy were previously reviewed and not accepted from MRID47901402 (DER attached).

III. MRID Summary:

1. The submitted studies were not done according to GLP.

2. Study Reviews

MRID 48556601 Muller, G.C. 2011. Efficacy Study: Evaluation of Toxic Attractive Sugar Bait in a Bait Station. The Hebrew University, Hadassah Medical School, Jerusalem, Israel.

Purpose: To determine the efficacy of the Westham HoneyTrap with its attractive toxic sugar bait to attract and kill mosquitoes.

Materials and Methods:

Test location: The Kuvin Centre for the Study of Infectious and Tropical Diseases, The Hebrew University, Hadassah Medical School. Jerusalem, Israel.

Test species: the adult life stage of the Yellow Fever mosquito, *Aedes aegypti*; the Northern House mosquito, *Culex pipiens*, and the African malaria mosquito, *Anopheles gambiae.* Mixed sex populations were used. Mosquitoes were never blood fed and were sucrose starved for 12 hours before testing. Mosquitoes were used only once.

Test substance: The treatment substance was identified as Westham HoneyTrap with its attractive toxic sugar bait (ATSB). The contents of the toxic sugar bait were described as 4.0% boric acid plus food grade attractants and Westham's Baitstub preservative. The positive control substance was 200 ml of 10% sucrose.

Laboratory Test System

Environmental conditions: 27° C; RH 80% and a 24 hour photoperiod of 16 hours of light: 8 hours of dark.

Experiment I.

Purpose: to demonstrate attractiveness of the HoneyTrap baited with ATSB compared to a conventional sugar source (10% sucrose).

Test chamber: The test chamber was an enclosed room that had dimensions of 4m x 4m x 2.8m. There were no windows and only a single entrance door. All surfaces were painted white. The lower part of the wall from 5-105cm from the floor was covered with a green rigid plastic fence. The floor was covered with a clean white linen sheet that was replaced for each experiment.

Modification of the HoneyTrap and the positive control: Attractiveness to the HoneyTrap and positive control was measured through the use of a trapping device. The device was prepared by rolling a 70cm x 70cm piece of stiff plastic mesh with 0.8 cm holes into cylinders. The cylinders were held fast by plastic tie wraps. Two cylinders were made. One cylinder was baited with a "honeytrap" and the other with a sponge soaked with 200 ml of 10% sucrose. The ends of the trap were covered with the same material use to make the cylinder and held in place with plastic ties.

Experimental procedure: Each experiment was species specific. Three hundred females and 300 males of the each species (600 in total) were released into the room at the same time. Mosquitoes acclimated to the room for 30 minutes. At 30 minutes a HoneyTrap baited trap and a sucrose baited trap were placed in different corners of the room to establish a choice test. The mosquitoes were exposed to the traps for 23.5 hours. Traps were collected at the end of this time whereby mosquitoes were sorted by sex and counted. Any mosquitoes remaining in the room were recovered with a power vacuum, sorted to sex, and counted. Choice test results were reported for each species replicate. Each trap type was rotated to each corner twice for a total of 8 repetitions per trap per species. New traps were used for each species specific experiment for a total of 8 devices for each treatment – 16 traps in total.

Experiment II.
Purpose: to demonstrate the palatability and kill efficacy of the ATSB when compared to a conventional sugar source (10% sucrose) over a 24 hour period in a no-choice test scenario.

Test substance and test species: The same trap types were evaluated as previously described. The same mosquito species were used and they were conditioned as described above.

Test chamber: Rectangular wire mesh cages measuring 120 cm x 60 cm x 60 cm with two sleeved openings that could be tied off to prevent mosquito escape.

Experimental procedure: three hundred females and 300 males of a single mosquito species were released into a cage. They were allowed to acclimate for 30 minutes. Either a HoneyTrap baited with ATSB or a trap baited with 10% sucrose was placed into the cage. Dye was added to the sucrose solution and the HoneyTrap that enabled the study director to identify the mosquitoes that had fed on the bait. Mosquitoes were allowed to feed over a 23.5 hour period. Dead and alive mosquitoes were collected separately and counted by sex. Each individual was examined for the presence of dye in their abdomen. New baits were used for each new cage of mosquitoes. For each treatment there were 10 replicates per species. Mosquitoes and baits were not reused.

Results:

Experiment I.

In each experiment a total of 2400 males and 2400 females of each species were released and tested. Pooled results are reported by species gender. Replicate specific results can be found in the study report.

Percent Attraction[2]

Species	*Culex pipiens*		*Aedes aegypti*		*Anopheles gambiae*	
Gender	M	F	M	F	M	F
ATSB[1]	96.08	94.17	90.04	90.42	93.17	91.83
10% sucrose	2.04	2.58	3.58	4.29	4.00	4.38
Not trapped	1.88	3.25	6.38	5.29	2.83	3.74

[1]HoneyTrap baited with ATSB = ATSB
[2]Percent attraction = total number captured in eight replicates/2400 mosquitoes of each sex

Experiment II.

Percent Fed and Percent Killed[2]

Species	*Culex pipiens*				*Aedes aegypti*				*Anopheles gambiae*			
Gender	M		F		M		F		M		F	
Efficacy	Fed	Killed	Fed	Killed	Fed	Killed	Fed	Killed	Fed	Killed	Fed	Killed
ATSB[1]	92.60	99.84	95.63	99.72	87.30	99.70	90.10	99.89	95.37	99.97	94.63	99.85
10% sucrose	88.37	0.15	86.77	0.19	83.97	0.16	84.53	0.08	90.43	0.55	92.00	0.33

[1]HoneyTrap baited with ATSB = ATSB
[2]Percent Fed/Killed = total number fed or killed in 10 replicates/3000 mosquitoes of each sex

Conclusion: The study is acceptable. The product is highly attractive and efficacious against the three important mosquito species when tested under laboratory conditions. It is effective against males and females of all three species.

MRID 48556602 Muller, G.C. and Y. Schlein. 2011. Efficacy Study: Different Methods of Using Attractive Sugar Baits (ATSB) for the Control of *Phlebotomus papatasi*. The Hebrew University, Jerusalem, Israel. J. Vector Ecology 36: S64-S70.

Purpose: This study tested the experimental use of ATSB application methods including vegetation treatment, treated barrier fencing and bait stations against sand flies.

Materials and Methods:

Test location: Central Jordan Valley Desert, Israel

Test species: field populations of the sand fly, *Phlebotomus papatasi.*

Test substance: The test substance had similarities to the product presented for registration, however, the inert ingredients are not all the same and not all of them are disclosed in this article. Furthermore, the study director added 0.4% spinosad to the formulation. Unlike boric acid, spinosad is well known for its ability to kill flies, especially fruit flies and house flies. The formulation was 95% over-ripened nectarine juice; 5% dry red wine; 10% w/v brown sugar; 0.5% w/v red food dye; 10% of a mixture of slow releasing substances (not identified) and preservatives (identified only as BaitStabH). The solution was allowed to ferment for 48 hours at temperatures of 30° C. The active ingredients, 1.0% w/v boric acid and 0.4% w/v spinosad were added after fermentation was complete.

The test substance was delivered as a residual spray to vegetation and barrier fencing as well as in a bait station.

Filed Test systems:

Six sites were selected for the experiment. Three were treated and three untreated. There was one treatment replicate for each ATSB application type. Each spray block had one treated and one untreated site. Each experimental site was 300 m long, 20-30 m wide and separated by 1 km from each other. Sand rats, which are the reservoir for *Leishmania major*, a causative agent of cutaneous Leishmaniasis and host of sand flies, were prevalent on all of the sites. Food dye present in the formulation was used as an indicator of sand fly feeding on the formulation in those sand flies caught in traps as described in the mentoring and evaluation section below.

Spray block 1 – Vegetation Treatment.
In the treated the bait spray was applied vegetation patches of 0.5 m in size. These spot treatments were made in an array to every fifth thicket of vegetation. This resulted in the treatment of between 10-20% of the standing vegetation on the site. Seven liters of ATSB solution were applied. The control treatment received untreated bait solution only.

Spray Block 2 – Barrier Fence Treatment.
First, the barrier fence was prepared and treated with ATSB. A semi-rigid plastic net that was 50 cm wide by 20 m long with a thickness of 2mm and mesh openings of 50 mm x 50 mm

was used the fence material. Strips of cotton cloth 5 x 60 cm in size were connected to the net. The process of connecting strips was repeated every 20 com until the fence material was covered. The fence with cloth strips attached was subjected to a dipping process to soak the cotton with ATSB solution or the solution without toxins. The ATSB solution was used in the treatment while untreated solution was used in the control. It was not clear from the article how much ATSB was applied or what the application intervals were on the barrier fence. The treated fence was staked into the ground with wooden stakes driven into the ground every five meters, which resulted in a barrier fence that was set.10 cm off the ground (to allow small animals/rodents to pass). Gaps of 1.5 meters were left every 25 meters to allow passage of larger animals.

Spray Block 3 – ATSB Bait Stations.
The same netting material was used in this experiment to construct bait stations as was used to make barrier fencing. For bait station construction the semi-rigid netting was cut into 50 cm lengths. Cotton cloth was affixed to the netting at 10 cm intervals. The resulting cotton covered net was rolled into cylinders and fixed with staples. Wooden stakes were used to hold the cylinders in a vertical position. Stations were set at distances of 10 meters apart. The number of stations was not stated. ATSB bait solution was sprayed on the treated site traps while bait solution without toxicant was applied to the untreated site traps.

Sand Fly Monitoring and Evaluation of Study Data:
Experiments were conducted from early June to the end of August. Sites were monitored before treatment every second day for ten days using non-baited CDC light traps (5 data points per site). Traps were hung 30 cm above the ground where sand flies are most active. After the treatment was applied, the sites were monitored every fifth day for 75 days (15 data points per site). The number of traps per site is not stated but the degrees of freedom in the experiment were quite high. During the post-treatment period the sand flies were examined for dye in their abdomen, which served as an indicator of bait feeding, noting that dyed solution was applied in treatments and controls. Treatment success was evaluated by comparing the male vs. female sand flies caught in the treatment compared to the untreated sites. Second, the pre-treatment versus post-treatment trap counts were compared using an unpaired one-tailed student's t-test.

Results:

The number of males compared to the number of females caught in the CDC light traps was not significantly different. Therefore, there appears to be no sex specific preference for bait solution. Only female sand fly data were presented in the results except for the barrier fencing where feeding percentages for males and females are presented.

Vegetation Treatment:

Sand fly populations were about the same in both sites before the treatment was made. Within one week of treatment, the sand fly population in the treated areas decreased dramatically and stayed suppressed throughout the experiment. The level of suppressions reached 94%. 60% of the flies caught in the untreated control fed on the untreated bait

solution as evidenced by dye in their abdomen.

Barrier Fence Treatment:

Sand fly populations in both sites were not significantly different before treatment except for one collection interval. Populations were the equivalent the following week at the site. The treatment showed an immediate effect and treated site sand fly populations stayed suppressed for the duration of the experiment. Percent reduction was about 88%. Sand fly feeding on the bait solution in the untreated control was 61.2% for females and 68.5% for males.

Bait Station Treatment:

Bait stations were the least successful method of treatment but the treatment differences were markedly different 3-4 weeks after the stations were made available. Bait station treatment result in a 60% reduction of sand fly populations. Feeding prevalence in the untreated control was 22.3% for males and 35.3% for females.

Conclusion: . This study is rated "partially acceptable" because the study director used a formulation containing two insecticides – spinosad and boric acid. Spinosad is known to be more toxic than boric acid to flies and a stand-alone field evaluation should have included a boric acid alone treatment. ATSB treatments were effective in sand fly reduction. Wide area barrier fence and vegetation treatments were most effective while bait station applications (using the design presented in this study) were least effective. The inert portion of the formulation is highly attractive to sand flies, but based on the study results, sand flies had difficulty finding the bait stations compared to applications made to vegetation or a barrier fence.

MRID48745201. Muller, G.C. et al. 2010. Control of *Culex quinquefasciatus* in a storm drain system in Florida using attractive toxic sugar bait. Medical and Veterinary Entomology, doi: 10.1111/j.1365-2915.210.00876.x

Purpose: To evaluate an ATSB against the Southern house mosquito, *Cx. quinquefasciatus*, in storm drains in Florida, USA.

Materials and Methods:

Test location: St. Augustine, Florida USA. The test plot area had no houses and was under development. Storm drain system had been installed. The system was 26 underground, interconnected storm drains that drained into 3300 m of drainage canal, which in turn, drained into three retention ponds. The system was associated with 1500 m of road. Storm drains were isolated and dry because they had been covered to prevent sediment drainage during development construction. There was no resident mosquito population breeding in the drains.

Test species: Southern house mosquito, *Cx. quinquefasciatus*.

Test substance: Attractive toxic sugar bait (ATSB) and untreated bait solution were the test

substances. The untreated base solution was prepared by mixing 95% juice from over-ripened plums with 5% w/v dry red wine. To this solution 10% brown sugar w/v; 10% w/v of a mixture of slow release substances and preservatives (BaitStab); and 0.5% w/v orange food dye. The entire solution fermented for 48 hours at 30° C. For the treated solution, 1.0% w/v boric acid was added and green food dye was substituted for orange dye to distinguish mosquito feeding in control and treatment sites.

Trap preparation: A 2 cm hole was drilled at about 2/3 of the way up of a 0.5 L soft drink bottle. A cotton wick was inserted in a manner that enabled the two ends of the wick to touch the bottom of the bottle, while the bend in the wick stuck out though the hole. The bottle was inserted into a clean white sock. The trap was dipped into the control or treatment solution and then filled with solution. The wick provided a constant supply of bait solution to the sock. An 18 cm dish shaped cover with an eyelet on top was fastened over the bottle. String of the needed length was tied through the eyelet. The traps were hung from drain covers so they were suspended in the drains above the sumps.

Test System:

38 L of water was added to each sump to create humid conditions. 500 ml of water containing 220 *Cx. quinquefasciatus* pupae were added to each sump. The design was not balanced and included 10 untreated drains and 16 treated drains. Drain covers were modified to allow mosquito to escape. At the surface, a small opening was covered with a conical exit trap to capture surviving adults. Conical traps were also placed at the drainage pipe exits. Six more beakers with pupae were kept outside the system to serve as another control for pupal emergence rates. This latter control accounted for any storm drain effect.

The experiment continued for eight days with daily collections made from all traps. Mosquitoes were counted and separated by sex.

Results:

The beaker controls provided a 93.5% adult emergence rate. 51.9% were male and 48.1% female. 5720 pupae were released into the drains. Based on the beaker control emergence rates, 2057 mosquitoes should have hatched in the 10 untreated drains while 3291 mosquitoes should have emerged in the treated drains.

Percent emergence and kill: Ninety percent of the mosquitoes expected were recovered from the untreated control drains. Only 18.7% were recovered from the treated drains. This translates into a treatment effect reduction of approximately 70-75%. There was no difference in efficacy based on sex of the mosquito.

Percent feeding: In the untreated sites, 88.2% of the females and 87.5% of the males fed on the bait solution. A small percentage of these escaped the untreated sites and penetrated the treated sites. On the other hand, mosquitoes did not penetrate the untreated sites from the treated sites. In the treated sites, where trap counts were much lower due to mosquito kill, 21% of the captured mosquitoes had fed on the bait while the rest had not. These mosquitoes

may have survived due to the slow toxic mode of action of boric acid.

The study directors also examined trap capture and feeding on a time basis. They concluded that the traps will be most effective if used at the time of pupal emergence because female mosquitoes seek a sugar meal before blood-feeding and males rely on a sugar diet and have a relatively short-life span. They also concluded that ATSB feeding may have affected behavior and mobility based on the lack of ATSB exposed mosquitoes in any of the untreated control traps.

Conclusion: The study is acceptable. The ATSB traps were effective at killing a population of *Cx. quinquefasciatus* mosquitoes, a major vector of St. Louis encephalitis and West Nile virus. This vector resides in storm water systems and sewers where control is difficult. ATSB based traps are an effective addition to the insecticide delivery methods for mosquito vector control, especially in urban and arid sylvatic habitats. The study supports the registration of the subject product.

IV. Entomologist's Recommendations:

1. Two of the studies are acceptable while the third is rated supplemental. As a result:

    a. An Asian tiger mosquito, *Aedes albopictus*, field study conducted in the USA is to be provided to EPA by October 31, 2013. The data for Asian tiger mosquitoes are to be collected by using the product as directed by the label. The data should also include population level monitoring over a three month period. Submit a protocol.
    b. A biting midge (from the family *Ceratopogonidae)* field study conducted in the USA is to be provided to EPA by October 31, 2013. The data are to be collected by using the product as directed by the label. The data should also include population level monitoring over a three month period. Submit a protocol.

2. Label
    a. The data support the addition of sand flies to the label provided biting midge study is conducted in the USA and the data are submitted by October 31, 2013. The addition of mosquitoes is acceptable to the label provided data on the Asian tiger mosquito, *Aedes albopictus*, are submitted by October 31, 2013.
    b. Submit a trap and refill cartridge as intended for use.
    c. The following information must be added to the label.
        i. Change the application rate to two traps per ¼ acre. There are no data to support a lower application rate.
    d. Remove the following claims, which appear on page 4, from the label:
        i. All "control" claims.
        ii. Proven effective floral lures attract sugar-feeding mosquitoes. Floral lures are not food-based attractants.
        iii. Patented formula kills mosquitoes in less than 24 hours after 1 feeding. (Despite the no-choice lab data provided, the field study did not support

this claim).

iv. Quickly reduces backyard mosquito populations. (Data were not provided to support this claim. Boric acid is slow killing).
v. Will not harm vertebrates (birds, animals, humans)
vi. Protective grid protects honey bees and other beneficial insects. (There are no data cited or provided to support this claim.)
vii. Remove the claims associated with the header "Call-outs".
viii. The size of the cartridge is not stated.
ix. Remove "professional use" claims
x. Remove the following claims from page 5 of the label:
    1. "Attracts and kills mosquitoes before they search for their first blood meal.
    2. Professional grade. Professional mosquito control.
    3. Patented formula attracts and kills mosquitoes.
    4. Patented.
    5. Odorless.
    6. Patented innovative control.
    7. Reduces mosquito populations over time.
    8. Slow release bait.
    9. Remove the term "Lures" from the label.
    10. Remove fast or quick kill from the label.
xi. Remove the following claims from page 6 of the label:
    1. Remove claims beginning with "Exclusive combination"
    2. Attracts [and kills] hard to catch species…
    3. The term [personal space]